Virchows Archiv A Pathol Anat (1993) 423:153–156

Virchows Archiv A
Pathological Anatomy and Histopathology

Editorial

Diffuse axonal injury: windows for therapeutic intervention allowed by its pathobiology

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Received April 22, 1993

Recognition of the significance of axonal transections in head-injured patients without other significant pathology began with the pioneering pathological studies of Strich, who in 1956 provided the first complete description of the lesion, which she termed "diffuse degeneration of white matter" (Strich 1956). Many subsequent studies have been published (Peerless and Rewcastle 1967; Oppenheimer 1968; Adams et al. 1977, 1982, 1989) which have extended Strich's initial observation and given rise to the concept of diffuse axonal injury (DAI) as the underlying pathophysiological substrate of diffuse brain injuries uncomplicated by secondary brain swelling or hypoxic/ischaemic damage. Two focal lesions that have been found to frequently accompany DAI and are considered indices of its severity are haemorrhagic lesions in the corpus callosum and in one or both dorsolateral quadrants of the rostral brainstem (Pilz 1983; Adams et al. 1989).

The pathology of DAI is variable and depends on the survival interval. With long survival (months to years), there is grossly apparent atrophy of the cerebral white matter with enlargement of the ventricles and widening of the cerebral sulci (Strich 1956). Myelin stains show diffuse pallor of the central white matter due to loss of myelinated fibres with relative preservation of the subcortical association fibres. With intermediate survival (weeks), there are large numbers of microglial scars or "stars" in the white matter tracts of the brainstem and forebrain. In acute to subacute survival (12 h to several weeks), the axonal retraction ball is the microscopic hallmark. This structure, which is also known as an axonal bulb, consists of a transected axon whose proximal stump has enlarged due to the continuing accumulation of the material of anterograde axoplasmic transport.

The axonal changes in DAI in the immediate (less than 12 h) post-traumatic period have been difficult to elucidate from clinical material, and clinical studies have been largely silent regarding this subject. Two large autopsy series investigating human DAI are in agreement that axonal retraction balls are not present until at least 12 h following the injury (Pilz 1983; Adams et al. 1989). One explanation for the absence of the pathological marker is that transection occurs instantly but is not detectable until sufficient anterogradely transported axoplasm has accumulated to form an enlarged bulb at the end of the axonal stump. However, an equally straightforward explanation is that the transection takes a number of hours to develop.

The changes in DAI in the 0 to 12 h period have been extensively investigated in experimental animals. These studies have shown that although axonal abnormalities are quickly detectable, actual transections are not immediately demonstrable even with quite severe impact (Erb and Povlishock 1988). Axonal abnormalities, termed reactive axonal changes and consisting, in the main, of progressively enlarging, irregular, focal swellings of the axon, precede the formation of actual transections by a matter of hours; retraction balls then become evident as the disrupted proximal axolemma reseals and continuing anterograde axoplasmic flow further enlarges the pathological stump (reviewed in Povlishock 1992). Recently, this remarkable and exciting result was found to pertain also to human material in a study using antibodies against the low molecular weight component of the neurofilament triplet (Grady et al. 1993).

The mechanism by which axons become progressively more varicose and ultimately severed is of great interest. Axotomy in the human CNS has been found to be functionally irreversible for all practical purposes and may, in and of itself, be fatal to the affected neuron (see below). The presence of a time interval between injury and transection argues for the existence of secondary mediators of axonal damage and suggests that therapeutic interventions to modify or abort the effects of the secondary mediators might be possible. Support for the existence of such a therapeutic window exists in the human literature of a closely related pathology, traumatic spinal cord injury. A very well-designed study has demonstrated that high dose methylprednisolone produces long term neurological benefit in this condition, but only when the initial dose of drug is given within 8 h of the injury (Bracken et al. 1990).

The list of potential secondary mediators of axonal transection in DAI is long, and many of the usual suspects have been or are being rounded up for investigation. The therapeutic effect of methylprednisolone in this setting requires such large amounts of the steroid that involvement of the steroid receptor cannot be the explanation. The investigations that led to the use of such a high steroid dose arose because animal studies of blunt trauma to spinal cord had shown beneficial effects of steroids through the drug-mediated scavenging of free radicals, typically oxygen-derived, and the prevention of lipid peroxidation (reviewed in Hall 1992). A class of drugs (21 aminosteroids) specifically designed for scavenging free radicals and preventing lipid peroxidation shows promise in the treatment of traumatic brain and spinal cord injury, and one such compound, tirilazad mesylate, is currently being used in clinical trials (Hall 1992). A common observation in the pathology of both human and experimental DAI is that the reactive axonal changes occur preferentially in those axons in close proximity to a vessel. This may be due to the purely mechanical effect of a pressure wave coursing along the vascular tree. However, bioactive substances that can be liberated by vascular cells, such as arachidonic acid metabolites (which are capable of generating oxygen-derived free radicals), appear interesting in this regard. In addition, there has long been evidence for a disorder of the blood brain barrier in experimental traumatic diffuse brain injury (Ommaya et al. 1964; Rinder and Olsson 1968). This might allow systemically-derived substances such as cytokines or neurotransmitters to penetrate into the brain from the vascular space. Interest has also been focused on the role that a physical stretching of the axon might play in producing abnormalities in either the plasma membrane or the cytoskeleton. One theory is that the stretching leads to an inability to maintain normal ionic gradients, especially calcium, in the paranodal region (Maxwell et al. 1991). This would lead, via the second messenger effects of raised cytosolic calcium, to the inappropriate activation of various enzymes such as proteases, which might ultimately contribute to the failure of axonal transport, and then to localized swelling and transection. Other investigations point to the cytoskeleton as a locus of vulnerability in DAI. Studies with anti-neurofilament immunohistochemistry (Yaghmai and Povlishock 1992; Grady et al. 1993) show a rapid "unmasking" of low molecular weight neurofilament antigen, while ultrastructural studies show a focal accumulation of organelles indicative of local loss of transport capability. A virtually immediate, stainable abnormality in the neuronal cytoskeleton has been demonstrated in traumatically injured rat brain (Gallyas and Zoltay 1992; Gallyas et al. 1992). Reactive axons were already demonstrable at one minute in the form of altered argyrophilia, which suggests that trauma may have an immediate and direct physical effect on the cytoskeleton, possibly analogous to triggering the release of a spring.

Devising pharmacological means to inhibit the progression of events leading to axonal transection would be a highly desirable strategy to limit the diffuse axonal damage in traumatic brain injury (TBI). Another strategy for which an interesting and logical scientific rationale exists is to keep alive the neurons whose axons have been transected. Such protection would open the possibility of later axonal regrowth and reconnection. Interestingly, not much has been written, in either the human or the experimental literature, about the actual loss of neurons in defined brain regions in DAI. Axotomy, the fundamental lesion of DAI, is known to cause pathological changes in neuronal cell bodies, which classically consist of either central chromatolysis or else simple atrophy. These alterations may proceed to cell death, depending on the degree of axonal loss, the maintenance of collateral projections, and other factors. The literature makes it clear that the signal for the pathological axotomy response is the loss of retrogradely transported neurotrophic factor. Studies with nerve growth factor (NGF), the paradigmatic neurotrophic substance, indicate that this factor is synthesized and released in limiting amounts by the target of the NGF-dependent neuron in question and that it is recognized by a receptor on the axon terminal and then internalized and retrogradely transported to the neuronal soma. In the well-defined experimental NGF-dependent systems, deprivation of NGF is equivalent to axotomy and vice versa, and provision of exogenous NGF to an axotomized NGF-dependent neuron prevents all of the pathological changes that can be demonstrated in the neuronal soma. Thus, the scientific rationale for the use of exogenous neurotrophic factors in DAI is much more straightforward than it is, for example, in any of the neurodegenerative conditions of unknown aetiology in which trophic factors have been proposed as a possible therapy, but in which the nature of the insult to the neuron is not clear.

There are many important questions that need to be answered in order to use neurotrophic substances rationally to improve neuronal survival in DAI. Even the successful application of neurotrophic substances will require the subsequent and probably enormously difficult development of therapies to facilitate functional neuronal reconnection. Saving damaged neurons from death would, nevertheless, seem to be a logical first step. The following questions, among others, will have to be addressed. What neurons need rescuing? What neurotrophic factor(s) do they respond to? How will the neurotrophic substances be delivered? The last of these questions is perhaps the most easily answered since intraventricularly administered NGF preserves axotomized septohippocampal cholinergic neurons in an experimental system (Hefti 1986; Kromer 1987), and CNS neurons generally appear capable of selective uptake of a variety of growth factors following intraventricular injection in the CNS of uninjured animals (Ferguson et al. 1991; Ferguson and Johnson 1991). However, the extent to which neurotrophic molecules can gain access to receptor-bearing neurons when such molecules are placed in the CSF in the traumatized CNS is not yet known. Molecular methods will probably be able to rapidly provide answers to the question regarding the trophic factor dependencies of various neurons by way of demonstrating which neurons express which receptors. The question as to which neurons need rescuing will require more scrutiny. As the name diffuse axonal injury implies,

the lesion tends to be diffuse, and the involvement of different functional neuronal systems is variable. Nevertheless, it seems reasonable to look for groups of neurons that are preferentially damaged in DAI, or at least to try to determine whether specific critical neuronal systems are compromised when the trauma to the CNS reaches a certain degree of severity. Two reasons that the fate of traumatized neurons in DAI has not to date been the focus of much study are: firstly, the detection of scattered or diffuse loss of neurons using standard pathological methods is extremely difficult; secondly, it has not been feasible to relate a group of axonal transections to the corresponding cell bodies. Providing the correlation between transected axons and injured somata would immediately allow many more investigations of the neuropathology of functional neural systems and would be a significant advance. Some recent discoveries about the nature of the cell death that axotomized or neurotrophic substance-deprived neurons undergo might be applied here. The death that axotomized or NGF-deprived superior cervical or dorsal root ganglion neurons are subject to has clearly been shown to be an active death of the sort that has been termed programmed cell death or apoptosis (Martin et al. 1988), and evidence has been produced for the generality of this phenomenon in the nervous system (Oppenheim et al. 1990). Programmed cell death is a distinctive process that has been well-studied in non-neural cell lineages (Wyllie 1987). It is distinguished morphologically by shrinkage of the cell body and by abnormalities in nuclear morphology that are then followed by cytoplasmic abnormalities. Biochemically, it is characterized by a requirement for active protein synthesis and by an endonuclease-dependent cleavage of the nuclear DNA into oligonucleosomal fragments of double-stranded DNA (Arends et al. 1990). In an in vitro NGF-dependent neuronal system which has been deprived of NGF there is a considerable period of time (18 h) before the neuron is irreversibly committed to undergo programmed cell death (Martin et al. 1988). This interval would appear to be another therapeutic window for saving neurons which have been axotomized or otherwise deprived of trophic factor. Since programmed cell death requires the co-operation of both the translational and the transcriptional apparatus of the injured cell, a gene or gene product might be found that will mark the injured neuron prior to its irreversible commitment to die. In the absence of knowledge of such specific gene products leading to programmed cell death, a recent study (Gavrieli et al. 1992) used the presence of fragmented DNA to identify apoptotic cells in a variety of well-studied systems of programmed cell death, such as lymphocytes and gut epithelium. The fragmented DNA could be found while both the nucleus and the cytoplasm of the cell still had a normal morphology. The data provided in this report are essentially correlative, and their applicability to neural systems has yet to be demonstrated. However, if the method could be extended to central nervous system neurons, it would be an especially useful technique for the histological evaluation of neuronal injury in DAI.

The foregoing discussion covers only a few of the many areas that offer opportunity in the study and potential treatment of DAI, and it is by no means meant to be a complete list. With this in mind, two questions logically arise. How important is it to explore the many avenues of promising DAI research? Is research in this area under-emphasized? To put the former question into proper perspective, one must realize that traumatic brain injury is a major world-wide health problem with estimates of annual incidence ranging from 200 to 400 cases/100,000 population in the industrialized nations and with 500,000 cases per year sufficiently severe to come to medical attention in the USA alone (Kraus 1993). Ten percent of these cases are immediately fatal, and many of the remaining 450,000 cases are ultimately fatal or cause serious long-term disability (Kraus 1993). Even in mild TBI, subtle alterations of higher cognitive function appear to be very common (Alves and Jane 1985) although they are not tabulated in most surveys. By far the most common form of TBI is closed head injury (Crompton 1985), with most of these cases being due to transportation-related accidents, or less often, falls. In addition to various focal injuries, closed head trauma frequently results in diffuse brain injuries such as brain swelling, hypoxic/ischaemic damage, and DAI, with DAI occurring in almost half of severely head-injured patients and being the cause of 35% of all deaths from head injury (Gennarelli et al. 1982). Almost two-thirds of all patients who die more than 1 day after head injury are shown to have DAI of some degree (Pilz 1983). DAI is also the greatest cause of severely disabled and vegetative survivors following head trauma (Graham et al. 1983).

With regard to the question of research emphasis, the resources allotted to head trauma research are best considered in the context of funding for trauma research generally, keeping in mind that head trauma is the most critical sub-category of trauma in terms of both morbidity and mortality. It is disappointing, therefore, that trauma research has been given a very low priority as measured by its level of funding relative to other health disorders (Hatziandreu et al. 1988; Campbell 1992). The reasons for this shortfall are multiple and include the fatalistic implications of the very word "accident" and the fact that the behaviour that leads to trauma is often regarded as something other than a health problem (e.g., a law enforcement problem). The relative under-funding of trauma research also reflects the fact that society has been slow to recognize trauma, especially TBI, as a public health problem. While the biggest public health benefits will logically come from research aimed at the prevention of TBI, DAI will continue to be a major cause of mortality and morbidity in a segment of the population that should be in the prime of life. Since scientific research in this area has uncovered windows for potential therapeutic intervention in what was once considered a hopeless condition, increased emphasis on DAI and other aspects of head trauma research is clearly warranted.

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